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# Mechanisms behind the polarized distribution of lipids in epithelial cells

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ABSTRACT

Epithelial cells are polarized cells and typically display distinct plasma membrane domains: basal plasma membrane domains face the underlying tissue, lateral domains contact adjacent cells and apical domains face the exterior lumen. Each membrane domain is endowed with a specific macromolecular composition that constitutes the functional identity of that domain. Defects in apical-basal plasma membrane polarity altogether or more subtle defects in the composition of either apical or basal plasma membrane domain can give rise to severe diseases. Lipids are the main component of cellular membranes and mechanisms that control their polarized distribution in epithelial cells are emerging. In particular sphingolipids and phosphatidylinositol lipids have taken center stage in the organization of the apical and basolateral plasma membrane domain. This short review article discusses mechanisms that contribute to the polarized distribution of lipids in epithelial cells.

## 1. Plasma membrane lipids

Lipids are the main component of cellular membranes [1]. Membrane lipid classes include glycerophospholipids, sphingolipids and sterols (Fig. 1) [2–4]. Glycerophospholipids contain a glycerol backbone and are the main structural lipids in eukaryotic membranes. The glycerophospholipid family includes phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and phosphatidic acid (Fig. 1). Sphingolipids contain a backbone of two long chain sphingoid bases, in eukaryotes dihydrosphingosine or sphingosine. Desaturation of the dihydroceramide base yields ceramide to which head groups can be added to form more complex sphingolipids, such as sphingomyelin (phosphocholine head group) and glycosphingolipids (various glucose residues) (Fig. 1). Many lipids are asymmetrically distributed between the inner and outer leaflet of the plasma membrane (Fig. 2), by virtue of lipid translocating enzymes (e.g., flippases, scramblases) and/or asymmetric lipid synthesis/metabolism [5]. Head group-containing sphingolipids are typically enriched in the outer (cell exterior-facing) leaflet of the plasma membrane, whereas phosphatidylethanolamine, -serine, -inositol and phosphatidic acid are typically enriched in the inner (cytoplasm-facing) leaflet of the plasma membrane [5]. Sterols, in mammals mostly cholesterol (Fig. 1), can be found in either leaflet.

## 2. The polarized distribution of (glyco)sphingolipids and cholesterol

The apical plasma membrane domain of epithelial cells is typically enriched in (glyco) sphingolipids (GSL) and cholesterol (Fig. 3; Table 1). Such lipid composition and the accompanied physicochemical properties are believed to allow the apical plasma membrane to serve as a robust and protective barrier. Shot-gun lipidomic analyses revealed that as epithelial cells develop apical-basolateral plasma membrane polarity, sphingolipids become longer, more saturated, more hydroxylated, and more glycosylated as indicated by increased amounts of galactosylceramide-sulfate and the globoside derivative Forssman glycolipid [2]. Acyl chain length, hydroxylation status and sphingolipid glycosylation all have been associated with the apical enrichment of sphingolipids [6,7]. GSL and cholesterol can form tightly packed (condensed) and at low temperature detergent-resistant membrane microdomains in the exoplasmic leaflet, known as lipid rafts. Lipid rafts are believed to develop as dynamic nanoscale entities that may coalesce to form larger microdomains within cell membranes [8]. Raft-based membrane heterogeneity has been proposed to allow sub-compartmentalization of both lipids and proteins in the membrane [8]. For example, the long saturated acyl chains of raft lipids may better accommodate proteins with relatively long hydrophobic transmembrane domains [8]. The ordered organization of raft lipids and the oligomerization of raft domains have also been proposed to promote local membrane phase separation which may promote membrane

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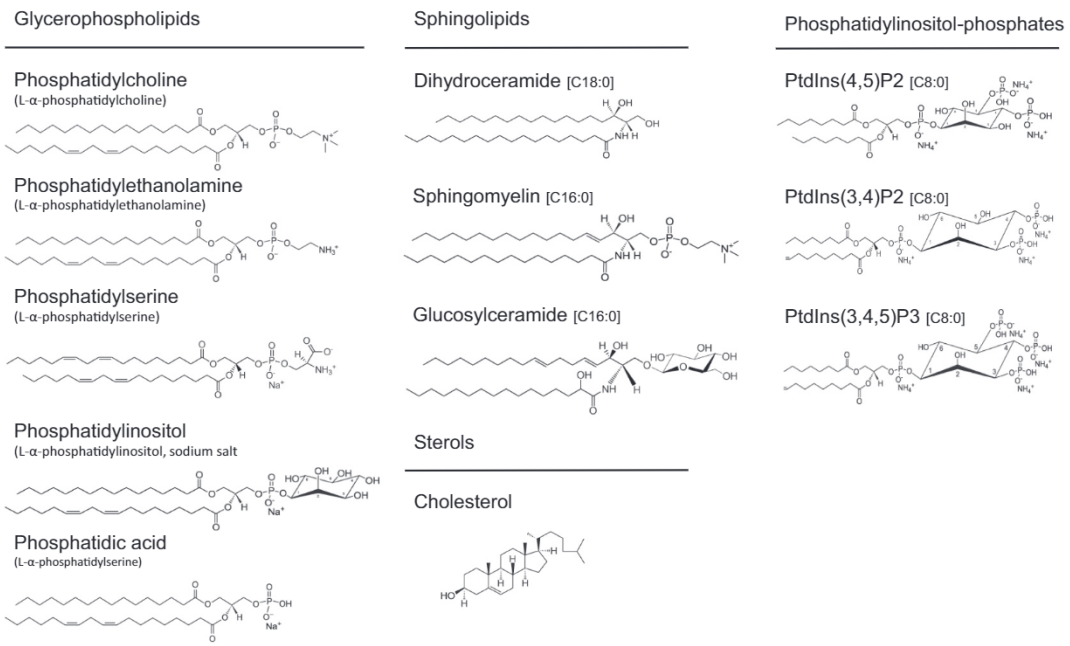



**Fig. 1.** Structures of the different lipids. The structures of glycerophospholipids, sphingolipids and phosphatidylinositol phosphates is depicted.

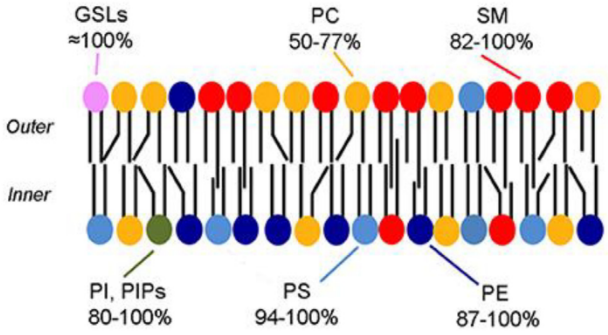
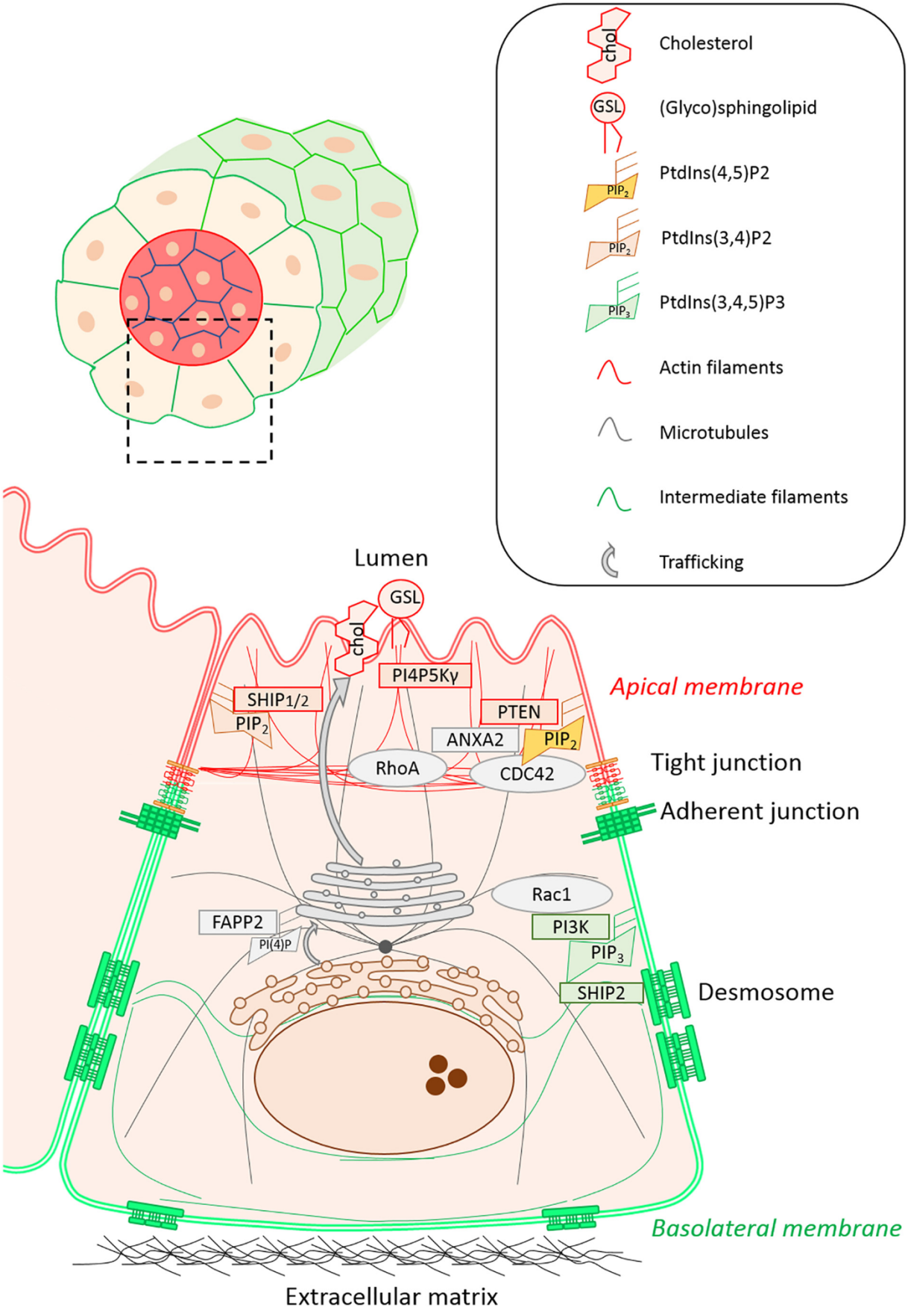



**Fig. 2.** Cartoon illustrating the asymmetric distribution of lipids in the lipid bilayer The majority of (glyco)sphingolipids is enriched at the outer (exoplasmic) leaflet of the membrane bilayer, whereas the majority of phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol lipids (PI, PIPs) are enriched at the inner (cytoplasmic) leaflet of the lipid bilayer.

curvature [9]. Lipid rafts may help to organize the apical plasma membrane into structurally distinct subdomains, such as the apical microvillar and inter-microvillar domains [10] and, in intestinal epithelial cells, deep apical tubules [11]. In intestinal epithelial cells cholesterol- and GSL-enriched lipid rafts may comprise up to 50% of the apical microvillar membrane domain [12]. Note that the concept of lipid rafts and their validation is subject to much debate of which a discussion is beyond the scope of this review, and the reader is referred to excellent review articles on this topic [8,13–15].

(Glyco)sphingolipids are mainly synthesized in the endoplasmic reticulum and in the Golgi apparatus. There are several ways via which cells can enrich GSL and cholesterol at their apical cell surface. First, the sorting and delivery of apical plasma membrane proteins and GSL is coupled [16,17]. It is believed that the delivery of many apical membrane proteins to the plasma membrane follows their intracellular sorting into small GSL- and cholesterol-enriched lipid rafts in the membrane of the *trans*-Golgi network, as evidenced by their cholesterol-dependent partitioning into detergent-resistant membrane fractions [9]. The concurrent clustering of these lipid rafts through the oligomerization (*i.e,* formation of high molecular weight complexes) of raft components facilitates the polarized sorting and targeting of apical membrane proteins and, concomitantly, the enrichment of these raft lipids at the apical surface [9,18,19]. The apical trafficking of the major apical GSL, Forssman antigen [20] (Table 1), from the Golgi apparatus to the apical surface was shown to require its interaction with the apically secreted galectin-9 [21]. Following its secretion, galectin-9 bound to the apically expressed and subsequently internalized Forssman antigen thereby forming a circuit between the Golgi apparatus and cell surface to facilitate the apical delivery of lipids and proteins [21]. The apical targeting of Forssman antigen was shown to be required for the development of the apical plasma membrane domain [21,22]. The extent of synthesis of the GSL glucosylceramide (GlcCer) appears critical for its own apical enrichment, as reduction of GlcCer synthesis resulted in the mislocalization of newly synthesized GlcCer at the lateral domain of epithelial cells in the *C.elegans* intestine [23]. Further, depletion of the phosphatidylinositol 4-phosphate (PtdIns(4)P) adaptor protein FAPP2 (four-phosphate adaptor protein), which mediates the transport of newly synthesized GlcCer from early to late Golgi membranes [24], caused the missorting of raft GSL and raft-associated proteins to the basolateral domain [25]. FAPP2 depletion also resulted in reduction of condensed lipid domains in the apical membrane and a concomitant increase in condensed domains in the basolateral membrane, as indicated by Laurdan staining [25]. GSL synthesis, galectin-9, PtdIns(4)P and FAPP2 thus regulate the polarized trafficking of GSL and contribute to the enrichment of lipid rafts at the apical plasma membrane domain. The presence of an apical recycling endosome system that is rich in sphingolipids and cholesterol [26] and the occurrence of lipid raft-based sorting of apical proteins in the endocytic system [27–29] is expected to support the apical enrichment of these lipids. Furthermore, Danielsen and colleagues reported that different fluorescent lipophilic probes when added to the apical plasma membrane of intestinal epithelial cells showed different rates of endocytosis, and it was suggested that lipid sorting by selective endocytic removal of non-raft lipids may help maintain the unique lipid composition at the apical domain [30]. Thus, the formation of oligomerizing GSL- and cholesterol-enriched lipid rafts and their selective sorting in the apical biosynthetic, endocytic and recycling pathways secure the enrichment of these lipids (and associated proteins) at the apical plasma membrane domain of epithelial cells.

Second, tight junctions (TJ) are since long considered as key structures that maintain the polarized distribution of proteins and of




*(caption on next page)*

**Fig. 3.** Cartoon illustrating the polarized distribution of lipids in polarized epithelial cells. Apicobasal polarity can be defined as symmetry breakdown within the epithelial cell, generating to poles: the apical pole (red) facing the lumen and the basolateral pole (green) facing adjacent cells and subjacent extracellular matrix. These two poles are separated by junction complexes composed by tight junctions, adherent junctions and desmosomes. Apicobasal polarity is maintained as a result of trafficking (grey arrows) polarity complexes (CRUMB, PAR and SCRIB complexes, not presented), Rho GTPases (CDC42, RhoA and Rac, presented as grey circle) and phosphatidylinositols PtdIns(3,4)$P_2$, PtdIns(4,5)$P_2$ and PtdIns(3,4,5)$P_3$. Other lipids are asymmetrically distributed within the polarized epithelial cell. Indeed, there is a specific enrichment of cholesterol (Chol) and glycosphingolipids (GSL) at apical membrane, contributing to lipid rafts composition. Legend is presented in black box.

**Table 1**
Table listing apical PM- and basolateral PM-enriched lipids. PM: plasma membrane, PtdIns: phosphatidylinositol; P: phosphate.

| Apical PM-enriched lipids | Basolateral PM-enriched lipids |
|---|---|
| Forssman antigen | PtdIns(3,4,5)P3 |
| Glucosylceramide | |
| Sphingomyelin | |
| Cholesterol | |
| PtdIns(3,4)P2 | |
| PtdIns(4,5)P2 | |

Table listing apical PM- and basolateral PM-enriched lipids. PM: plasma membrane, PtdIns: phosphatidylinositol; P: phosphate.

sphingolipids, but their role in this has been challenged. TJ are strategically positioned at the boundary between apical and basolateral domains where the TJ transmembrane proteins (occludin, claudins and junction-associated membrane protein (JAM)-1) form homotypic protein-based interactions between neighboring cells [31]. These homotypic interactions limit the extracellular space between the plasma membranes of neighboring cells such that plasma membrane proteins or lipids with relatively bulky ectodomains may be prevented from laterally diffusing from the apical to the basolateral membrane domains. The apical confinement of lipids that are present in the outer - but not inner - leaflet of the plasma membrane was demonstrated with the use of fluorescently labeled lipids, and this confinement was lost after perturbing cell-cell adhesion with extracellular calcium chelators [32]. This observation has been the prime foundation for the hypothesis that cell-cell adhesions and in particular TJ secure the polarized distribution of sphingolipids. However, the transmembrane TJ protein claudin-4, when reconstituted into giant unilamellar vesicles, formed adhesive membrane interfaces and led to the partitioning of ectodomain-containing membrane proteins, but not of lipids present in either outer or inner leaflet [33]. Bellardi and colleagues suggested that junctional strands formed by TJ-associated proteins may be required for the intramembranous lipid barrier [33]. However, the expression of a COOH-terminally truncated occludin protein resulted in the inability of epithelial cells to retain fluorescent lipids in either apical or basolateral cell surface domain, while junctional strands appeared unaffected [34]. Notably, the depletion of occludin from epithelial cells did not affect the confinement of fluorescent proteins and lipids to either apical or basolateral domain. Even the complete absence of functional TJ (by deleting tight junction-associated zona occludens (ZO)-1, -2, and -3) proteins) did not lead to the redistribution of lipids from the apical to the basolateral surface domain, as assessed with apically inserted fluorescent lipid analogues and with the sphingomyelin-binding lysenin protein [35]. Hence, the precise role of TJ in maintaining lipid polarity remains elusive.

Sphingolipid-cholesterol rafts are preferred platforms for membrane-linked actin polymerization [36] The association of lipid rafts with other apical membrane proteins and with cortical cytoskeletal proteins can form macromolecular scaffolds which, in turn, may reinforce the enrichment of raft lipids at the apical cell surface. For example, clustering of sphingomyelin in the apical domain correlated with the Par6-mediated formation of actin-based filamentous structures, rather than with the establishment of functional TJ [35]. Single-molecule imaging of fluorescently labeled cholesterol in the plasma membrane of non-polarized cells suggested that lipid raft domains coexist with membrane-skeleton-induced compartments and are contained within them [37]. Neurons do not have TJ yet are also polarized cells with axons and neurites representing the equivalent of apical and basolateral domains in epithelial cells. In these cells, the accumulation of membrane proteins anchored to the dense membrane skeleton at the axonal initial segment was shown to function as a diffusion barrier for the unsaturated phospholipid L-α-dioleoylphosphatidylethanolamine between the axonal and neurite plasma membrane domains [38]. Conceivably, the extensive cortical cytoskeleton networks beneath the apical plasma membrane of epithelial cells may likewise provide boundaries (fences) that limit the lateral diffusion of membrane lipids. Of interest, functional mutations in the small GTPases RhoA, Rac1, and Cdc42, which are heavily involved in (subapical) cortical actin organization, have been reported to perturb epithelial sphingolipid polarity [39,40].

## 3. From outer to inner leaflet lipid composition

The organization of lipids in the exoplasmic leaflet of the apical plasma membrane may also regulate lipids in the inner leaflet. For example, the clustering of sphingomyelin at the apical plasma membrane domain of epithelial Eph4 cells mediated the co-clustering of the transmembrane sialoglycoprotein podocalyxin-1. Subsequent binding of the scaffolding protein sodium-hydrogen exchanger regulatory factor 1 (NHERF1/EBP50) to podocalyxin-1 recruited phosphatidylinositol 4-phosphate 5-kinase (PI4P5K)-beta (but not -alpha or -gamma) [41]. This lipid kinase phosphorylates the phosphatidylinositol lipid phosphatidylinositol (4)-phosphate (PtdIns(4)P) to form phosphatidylinositol (4,5)-bisphosphate (PtdIns(4,5)P2), which was earlier reported to be enriched in lipid raft fractions isolated from epithelial cells [42]. In agreement, GFP–PLCδ-PH, which is known to bind to PtdIns(4,5)P2 specifically, localized to the apical plasma membrane in a sphingomyelin-dependent manner [41] (Fig. 3). Also in the plasma membrane of the cleavage furrow in dividing cells, super-resolution fluorescence microscopy revealed transbilayer colocalization of sphingomyelin-rich domains in the outer leaflet and PtdIns(4,5)P2-rich domains in the inner leaflet, the latter of which were lost upon sphingomyelin depletion [43]. Thus, the accumulation and clustering of sphingolipids in the outer leaflet of the apical plasma membrane may - likely via co-clustered transmembrane proteins - act as a cue for the recruitment of PtdIns kinases and subsequent synthesis and enrichment of specific phosphatidylinositol lipids in the opposing inner leaflet of the apical plasma membrane.

## 4. The polarized distribution of phosphatidylinositol lipids

Phosphatidylinositol lipids are glycerophospholipids containing a glycerol backbone, two fatty acid tails and a phosphate group substituted with an inositol polar head group (Fig. 1). Phosphorylation and dephosphorylation rates of the 3-, 4-, or 5-position of their inositol ring by the concerted action of cytoplasmic kinases and phosphatases produces a dynamic variety of phosphoinositides with distinct functions. Phosphoinositides are at the basis of complex polarized cortical protein networks that have emerged as critical determinants of epithelial cell polarization and of apical and basolateral membrane identity [44]. Indeed, though the use of fluorescent protein-fused PtdIns-binding domains [45], phosphatidylinositol lipids have been shown to adopt a

polarized distribution in epithelial cells as PtdIns(4,5)P2 ([46], PtdIns (3,4)P2 [47] and PtdIns(3,4,5)P3 [48] are enriched at the cytosolic leaflet of the apical and basolateral domains, respectively (Fig. 3; Table 1). As the different phosphatidylinositol lipids are typically produced by cytosolic phosphoinositide kinases and phosphatases, it follows that the polarized localization and concerted activities of these enzymes and their lipid substrates dictate the apical versus basolateral enrichment of these lipids.

## 5. Phosphatidylinositol lipids at the apical plasma membrane

PtdIns(4,5)P2 has been demonstrated to be enriched at the apical plasma membrane domain of epithelial cells (Fig. 3; Table 1). The de novo development of the apical plasma membrane is conveniently studied using epithelial MDCK cell which, when grown in three-dimensional (3D) culture in an extracellular matrix, form monolayered hollow cysts with their basolateral plasma membrane facing the extracellular matrix and the apical plasma membrane facing a fluid filled central lumen [49]. PtdIns(4,5)P2 is detected at the nascent apical domain concomitant with the 3′ lipid phosphatase PTEN (phosphatase and tensin homolog deleted on chromosome 10) which can produce PtdIns(4,5)P2 from PtdIns(3,4,5)P3. Knockdown of PTEN from these MDCK cells effectively prevents the de novo formation of the apical plasma membrane domain and that of the central lumen, presumably by preventing the conversion of PtdIns(3,4,5)P3 to PtdIns(4,5)P2 [46] or, as recently proposed, by preventing the local depletion of PtdIns (3,4,5)P3 [47]. Interestingly, the ectopic insertion of PI(4,5)$P_2$ into the basolateral surface of epithelial cells resulted in the formation of apical patches in the basolateral domain containing apical resident proteins. These data led to the proposal that PtdIns(4,5)P2 is a determinant of apical plasma membrane identity [46].

Also in Drosophila epithelial cells, PtdIns(4,5)P2 is enriched at the apical plasma membrane [50,51]. Interestingly, the enrichment of PtdIns(4,5)P2 at the apical plasma membrane in these cells required the PI4P5K Skittles (SKTL) but not PTEN [50]. Inactivation of SKTL led to constriction of the apical plasma membrane and a concomitant change in cell shape. It was proposed that the PI4P5K SKTL, by promoting the apical enrichment of PtdIns(4,5)P2 regulated the size of the apical domain and thereby contributed to the dynamic remodeling of the epithelial tissue [50]. Indeed, prior to the apical constriction in a context of endogenous epithelial invagination, PtdIns(4,5)P2 was found to be depleted from the cell apex [50]. In Drosophila epithelial cells of the salivary gland, loss of the apical polarity protein Crumbs resulted in increased apical levels of PtdIns(4,5)P2 in a PTEN-dependent manner [51], and the formation of microvillus inclusion bodies [51] reminiscent of those seen in intestinal epithelial cells of patients diagnosed with microvillus inclusion disease [52,53]. Also in intestinal epithelial Ls174T-W4 cells, which develop apical-basolateral plasma membrane polarity as single cells, PtdIns(4,5)P2 was shown to accumulate at the apical plasma membrane [54]. However, in contrast to MDCK epithelial cells, in these cells the loss of PTEN resulted in the expansion of the apical plasma membrane domain [55]. In Drosophila embryonic epithelial cells, inhibition of PI3K, which is expected to prevent the conversion of PtdIns(4,5)P2 to PtdIns(3,4,5)P3, results in apical membrane growth [56].

PtdIns(4,5)P2 is not exclusively localized at the apical plasma membrane and a recent study challenged the role of PtdIns(4,5)P2 as the (sole) apical plasma membrane determinant [47]. Instead, using EGFP-2xPH-TAPP1 as a reporter, PtdIns(3,4)P2 was shown to be enriched at the apical plasma membrane of polarized MDCK cells in 3D culture and proposed to act as a key determinant of apical plasma membrane identity [47]. PtdIns(3,4)P2 is mainly generated by the dephosphorylation of PtdIns(3,4,5)P3 by the Src homology 2 (SH2) domain/containing inositol polyphosphate 5-phosphatase 1 and 2 (SHIP1 and SHIP2). SHIP1 was found to be transcriptionally upregulated, and to dynamically relocalize from the basolateral to the apical plasma membrane shortly after the formation of the lumen, and inhibition of SHIP1 expression resulted in an increase in basolateral PtdIns(3,4,5)P3 [47]. Two other 5-phosphatases that might produce apical PtdIns(3,4) P2 using PtdIns(3,4,5)P3 as substrate, INPP5E (inositol polyphosphate 5-phosphatase E) and OCRL (INPP5F; mutated in patients with Lowe syndrome), were not observed at the nascent apical plasma membrane of MDCK cells and, therefore, are not likely to contribute to the apical enrichment of PtdIns(3,4)P2 [47]. However, INPP5E was found to be recruited to cilia, which are specialized subdomains of the mature apical plasma membrane [57]. Furthermore, 4-phosphatases INPP4A/B, which can convert PtdIns(3,4)P2 to PtdIns(3)P, were initially downregulated in 3D MDCK cultures, in this way likely contributing to the apical enrichment of PtdIns(3,4)P2. The recruitment of SHIP1 to the developing apical plasma membrane may be needed only for the initial apical enrichment of PtdIns(3,4)P2 (when there may still be PtdIns (3,4,5)P3 present) in 3D cultures. Hereafter, the apical enrichment of PtdIns(3,4)P2 is likely reinforced by the apical delivery of rab11a vesicles in which class-II PI3-kinases PIK3C2A/B convert PtdIns(4)P to PtdIns(3,4)P2 [47]. Interestingly, other studies [58,59] reported a predominant basolateral localization of PtdIns(3,4)P2, and its producing enzyme SHIP2 in polarized MDCK cells, where it was proposed to bind the evolutionary conserved basolateral polarity protein Dlg1 and recruited its complex partner Scribble to the basolateral plasma membrane. In addition to its basolateral localization, PtdIns(3,4)P2 was also detected at the apical plasma membrane of polarized MDCK cells but in specific domains that corresponded to cilia [59], and the lipid phosphatase activities of SHIP2 and INPP5E play an important role in ciliogenesis [60,61]. Conceivably, the polarized distribution of PtdIns (3,4)P2 in epithelial cells is not fixed, controlled by the concerted actions of SHIP1, SHIP2, PIK3C2A/B and vesicular PtdIns(3,4)P2 supply, and may change during the dynamic processes of cell surface remodeling.

The functions of phosphoinositide lipids at the apical plasma membrane have been the topic of excellent recent reviews [44,62–69]. PtdIns(4,5)P2 but, not PtdIns(3,4)P2, at the nascent apical plasma membrane recruits components of the apical membrane trafficking machinery, including annexin-2, the small Rho family GTPase cdc42 and rab11a-positive recycling endosomes [46]. The apical enrichment of PtdIns(4,5)P2 in single polarized Ls174T-W4 cell was followed by the recruitment of the cytosolic phospholipase D1. Phospholipase D1 converts phosphatidylcholine in the cytoplasmic leaflet to phosphatidic acid, and this allowed for the polarized accumulation of phosphatidic acid at the apical plasma membrane. The negatively charged phosphatidic acid, in turn, provided a local cue for the successive recruitment of the small G protein rap2A, its effector TNIK, the kinase MST4 (which partly localizes to rab11a-positive apical recycling endosomes [70]) and, ultimately, the MST4-mediated phosphorylation and activation of the actin-binding protein ezrin [54]. Of interest, phosphatidic acid may participate in the regulation of PI4P5K required for the synthesis of PtdIns(4,5)P2, and in this way provide a positive feedback loop. PI4P5K and PI(4,5)$P_2$ also contributes the organization of the subapical cortical actin cytoskeleton by facilitating the binding and activation of ezrin [65,71,72], which is a pivotal step in the development of apical microvilli. The production of PtdIns(3,4)P2 at the apical domain may provide a zone for apical exocytosis and, via recruitment of sorting nexin 9 (SNX9), stimulate endocytosis of basolateral proteins at the nascent apical plasma membrane [47].

In conclusion, the apical recruitment and/or activity of the 3′ and 5′ phosphatase PTEN and SHIP1 the lipid kinases PI4P5K and apical recycling endosome-associated PIK3C2A/B allow for the exclusion of PtdIns(3,4,5)P3 and enrichment of PtdIns(4,5)P2 and PtdIns(3,4)P2 at the apical plasma membrane. An outstanding question concerns the mechanisms that drive the polarized recruitment of these lipid kinases and phosphates and the feedback loops that are needed to reach a steady-state equilibrium.

## 6. Phosphatidylinositol lipids at the basolateral plasma membrane

In polarized epithelial cells, PtdIns(3,4,5)P3 is typically excluded from the apical plasma membrane and stably located at the basolateral plasma membrane (Fig. 3; Table 1), as shown by the localization of Akt pleckstrin-homology binding domain fused with GFP (GFP-PH-Akt), a fusion protein that binds to PtdIns(3,4,5)P3 [48,73]. The ectopic addition of PI(3,4,5)$P_3$ at the apical plasma membrane of polarized MDCK cells induced the formation of protrusions containing a basolateral membrane protein and excluding apical membrane proteins [48], indicating that this phosphoinositide controls basolateral membrane identity. Not much is known about the mechanisms that restrict PtdIns (3,4,5)P3 to the basolateral surface. The basolateral distribution of PtdIns(3,4,5)P3 may be a simple reflection of the exclusion of PtdIns (3,4,5)P3 from the apical plasma membrane by 3′ and 5′ phosphatases, possibly also involving the activity of the atypical protein kinase C (PKC)-lambda [74]. In addition, it may be result of the basolateral production of PtdIns(3,4,5)P3. Heterodimeric class I PI3 kinases (PI3K) are the major enzymes producing PtdIns(3,4,5)P3 from PtdIns(4,5)P2. There are four mammalian class I PI3K isoforms consisting of a common regulatory subunit and a specific catalytic subunit p110α, β, γ, δ for PI3K α, β, γ, δ, respectively. Of these, the catalytic subunit PI3Kδ was reported to localize to focal adhesion sites at the basolateral plasma membrane of polarized MDCK cells in a catalytic activity-dependent manner [75], where it thus may contribute to the production of PtdIns (3,4,5)P3. In support, the pharmacological pan-inhibition of class I PI3K in MDCK cells reduced basolateral plasma membrane size with abnormally short lateral surfaces and, thereby, the height of the epithelial monolayer [48,76]. Basolateral PI3K activity regulates laminin and collagen-IV assembly at the basolateral surface [75], while these extracellular matrix proteins in turn can activate PI3K [77], establishing a feedback loop that could reinforce basolateral PI3K activity and PtdIns (3,4,5)P3 enrichment.

Interestingly, it was shown that the bacterium and human pathogen *Pseudomonas aeruginosa*, when bound to the apical surface, recruited and activated PI3K to the apical plasma membrane. As a result, at the site where the bacterium attached to the apical plasma membrane local PtdIns(3,4,5)P3-enriched membrane protrusions were formed that contained basolateral resident proteins and allowed cellular entry of the bacterium. Thus, *Pseudomonas aeruginosa*, by modulating the polarized distribution of phosphoinositides created a local microenvironment that facilitated its colonization and cellular entry [78]. Moreover, these data further support that localized PI3K activity contributes to PtdIns(3,4,5) P3 enrichment.

Wang and colleagues [79] exploited this phenomenon to stimulate the uptake of nanoparticles into the polarized epithelium. Delivery of drug-carrying nanoparticles to their site of destination is a major hurdle in modern medicine [80] The uptake of nanoparticles into polarized epithelia via the apical plasma membrane is prohibited, presumably because the integrin receptors that facilitate their uptake are localized at the opposing basolateral plasma membrane domain [81]. Wang and colleagues therefore produced PtdIns(3,4,5)P3-containing nanoparticles carrying siRNA against a host cell gene product. It was demonstrated that these nanoparticles delivered the PtdIns(3,4,5)P3 into the apical plasma membrane domain, which resulted in a local conversion of the apical membrane into a basolateral domain containing the basolateral integrin receptors, followed by the uptake of the nanoparticles into the cells and silencing of the targeted host gene [79].

In conclusion, the polarized distribution and activity of PI3K to the basement membrane-exposed basolateral domain, concomitant with the polarized distribution and activity of 3′ and 5′ phosphatases at the apical domain, likely dictate the polarized distribution of PtdIns(3,4,5) P3 at the basolateral plasma membrane.

## 7. Concluding remarks

Lipids, being the main component of cellular membranes, and in particular (glyco)sphingolipids and phosphatidylinositol lipids, have taken a center stage in the organization of the apical and basolateral plasma membrane domain. Different mechanisms contribute to the polarized distribution of these lipids in epithelial cells which, depending on the type of lipid, may include polarized lipid trafficking, local lipid retention and/or local lipid synthesis and metabolism. Recycling endosomes have emerged as key regulators of polarized lipid distribution, and it will be of interest to determine the contribution of lipids to diseases where loss of recycling endosome function causes polarity defects, such as microvillus inclusion disease. Also of interest for future studies is the reported link between outer leaflet sphingolipids and inner leaflet phosphoinositide lipids, which have long been studied separately. Future studies may also be directed at the mechanisms that drive and integrate the transcriptional regulation of phosphoinositide lipid producing enzymes, their polarized recruitment and polarized phosphoinositide lipid profiles.

## Declaration of competing interest

The authors declare no conflict of interest.

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